Quantitation of Steryl Ferulate and p-Coumarate Esters from Corn and Rice[1]

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ABSTRACT: The principal steryl ferulate and *p*-coumarate esters of different fractions from processed corn brans and corn oils, unrefined and refined, and from rice bran and rice bran oil were quantified by high-performance liquid chromatography. The results show that hexane-extracted corn oils yield more than five times the amount of esters compared to expeller processed oils. The yields of esters from bran and related products ranged from 0.07 to 0.54 mg/g of bran. Unrefined corn oils had levels from 0.18 to 8.6 mg/g for oil from hexane-extracted bran. By comparison, rice bran had ester levels of 3.4 mg/g of bran, and rice bran oil had levels of 15.7 mg/g of oil. The predominant esters from corn were sitostanyl and campestanyl ferulate, and sitostanyl and campestanyl *p*-coumarate. The principal esters from rice bran were cycloartenyl, 24-methylenecycloartanyl, and campesteryl ferulate. Rice bran oils had low levels of 24-methylenecycloartanyl but high levels of cyclobranol esters. The data presented provide a direct comparison of steryl ferulate and *p*-coumarate levels in the two cereals, and will aid in selecting the most suitable sources for the isolation of these compounds from corn products.
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Sterols esterified to cinnamic acid and its derivatives (CAD) are found in cereals and other plant families (1,2). The ferulates of cyclic dimethyl and desmethyl sterols occur in rice (3) and are collectively termed γ-oryzanol. Ferulate and *p*-coumarate esters of desmethyl sterols are found in corn, rye, wheat, and triticale (1). Two aspects of steryl CAD esters in corn and related cereals are of interest, namely the possible involvement of these compounds in the susceptibility, or resistance, of preharvest corn to pathogens and whether these compounds might have effects similar to those of rice bran oil or γ-oryzanol on certain types of hypercholesterolemia.

No specific function for these compounds in the plant has been demonstrated, however, one possibility is that they may affect the response of the seed to pathogens, either positively or negatively. The compounds are localized in corn kernels in the inner pericarp and aleurone layer (1), and ferulic acid, the principal compound esterified to the sterols, has been shown to inhibit growth and aflatoxin production in *Aspergillus flavus* (4,5), which is the primary source of aflatoxin contamination in preharvest corn (6). It was anticipated that hydrolysis of the ester by infecting fungi would release inhibitory levels of ferulic acid within the region of the kernel in which they occur. Recent evidence indicates that steryl CAD esters increase the amount of aflatoxin produced by *A. flavus* grown on a synthetic medium but did inhibit spore germination of *Scerlotinia sclerotiorum*, which is not a pathogen of corn but is a pathogen in other plants (R. Norton, and P. Dowd, unpublished data).

Rice bran oil has been reported in a number of studies to be effective in lowering blood cholesterol in some types of hypercholesterolemia, and γ-oryzanol has been suggested as the active factor (7). Similar studies have not been reported on this ester fraction from cereals other than rice. The levels of steryl CAD esters from intact whole corn were low compared with those from rice bran oil, i.e., about 46 μg/g for corn kernels (1) vs. 3500 μg/g for rice bran (8). Localization of the esters in the pericarp and aleurone layers indicated that bran could be a more efficient source of these compounds than whole corn and a recent report showed that one corn bran product contained about twice the level of the steryl CAD as did whole corn, and it contained at least 16 identifiable steryl esters (9). Even a doubling of the concentration of esters by using bran would still give a low yield for corn compared to rice. Because the pericarp and aleurone layers comprise most of the bran fraction, but make up only about 5% of the kernel (10), a yield of 20 times the kernel level would seem to be the upper limit, i.e., about 900 μg/g of bran. To determine which corn products might be the best source for these compounds, it would be helpful to obtain enough of the 16 or more minor constituents of bran for testing against corn pathogens as well as for testing their efficacy in the treatment of hypercholesterolemia.

In the studies reported here, we analyzed different bran fractions for composition and yield of steryl CAD esters and compared these to data obtained on unrefined and refined corn oils. The composition and levels of esters in rice bran and rice bran oil have been reported by others (7,8,11), but samples of

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Abbreviations: CAD, cinnamic acid derivative; CV, coefficient of variation; HPLC, high-performance liquid chromatography.

these products were also included in the present study to permit direct comparisons of data obtained by the same analytical methods. Nothing is known to date about the biosynthesis of these compounds. It would also be of interest to know whether esters from different parts of the corn kernel have similar ester levels and compositions. The specific ester compositions of the fractions would be important for evaluating the physiological effects of these compounds from corn in comparison to those from rice.

MATERIALS AND METHODS

Corn bran fractions designated raw bran, bran duster thrus, bran after duster, finished bran, fine high-fiber corn bran (No. 97800), standard corn bran (No. 96530), and ultra-high fiber corn bran (No. 99030) were obtained from Illinois Cereal Mills (Paris, IL). Corn oil processing intermediates designated expeller cake, crude corn oil (expeller extracted), and crude corn oil (hexane-extracted) were obtained from Lauhoff Grain Co. (Danville, IL). Commercial oils and rice bran from the following manufacturers were obtained from local health food stores: Spectrum Naturals, Inc. (Petaluma, CA); Select Origins Inc. (Southampton, NY); California Naturals (The California Olive Oil Corp., Salem, MA); BioSan (New Dimensions Distributors, Fountain Hills, AZ); NOW Foods (Glendale Heights, IL); Hain Pure Food Corp. (Los Angeles, CA); Mazola corn oil (CPC International Inc., Englewood Cliffs, NJ). γ-Oryzanol (as γ-Oryzanol), was purchased from CTC Organics (Atlanta, GA).

Extractions. Aliquots of bran and expeller cake were weighed and dried at 100°C to constant weight to determine percent moisture. Duplicate samples equivalent to 50 g of dry weight were mixed with 250 mL hexane, allowed to set, and extracted twice overnight in the dark. The samples were filtered through a Büchner funnel with filter paper, and the cake was rinsed twice with 125 mL of hexane. The combined extracts were evaporated under reduced pressure at 40°C and stored at −20°C along with the commercial oils prior to use.

Sample preparation. Free fatty acids were removed from samples by adding 3 mL of 1,2-dichloroethane (Sigma, St. Louis, MO) to a 1-g sample of the oil obtained by hexane extraction of bran or the oil obtained from the manufacturer. Next, 1.5 mL of a saturated aqueous solution of Na_2CO_3 (40%, wt/vol) was added to the diluted sample and mixed thoroughly. After centrifuging the mixture on a laboratory centrifuge for several minutes, the organic layer was pipetted off. The aqueous phase was extracted with an additional 3 mL of 1,2-dichloroethane, and the combined organic phases were evaporated to dryness with a stream of N_2 at ambient temperature (12). The dry extract was redissolved in 1 mL of hexane/Et_2O (9:1, vol/vol). A silica gel column was prepared by adding 10 g of silica gel (63–200 μm mesh; Alltech Associates, Deerfield, IL) made up in hexane/Et_2O (9:1, vol/vol) to a 50-mL glass column (28 × 100 mm; Kontes Glass, Flex-Column, Vineland, NJ). The sample was pipetted onto the column and eluted with Solvent Systems S_1, i.e., 50 mL hexane/Et_2O (9:1, vol/vol); S_2, i.e., 30 mL hexane/Et_2O (7:3, vol/vol); and then S_3, i.e., 50 mL hexane/Et_2O (5:5, vol/vol). The final 10 mL of S_2 and the entire volume of S_3 were collected as 10-mL fractions. The fractions were evaporated under reduced pressure and brought up to 1 mL with 2-propanol. The presence or absence of ferulates and *p*-coumarates in the column eluates was monitored by measuring absorption at 327 nm, using a Beckman DU 60 spectrophotometer (Beckman Instruments, Fullerton, CA). Triglycerides, sterol fatty acid esters, and fatty acids were eluted in S_1 and in the first 20 mL of S_2. Steryl CAD was eluted in the last 10 mL of S_2 and S_3. Sterols were not removed, but these were eluted ahead of the steryl CAD esters and did not absorb at the wavelength used for monitoring esters. Examination of all fractions eluted from the silica gel column showed that no appreciable amounts of esters were lost due to premature elution or due to incomplete elution.

High-performance liquid chromatography (HPLC). The steryl CAD mixture obtained was analyzed by HPLC using a C18 reversed-phase (5 μm, 300 Å, Deltabond ODS; Keystone Scientific, Bellefonte, PA) column (4.6 × 250 mm) and acetonitrile/*n*-butanol/acetic acid/water (82:3:2:13, by vol) as solvent system at a flow rate of 1.2 mL/min at ambient temperature. The effluent was monitored using a diode-array detector at 325 nm. Corn components were identified spectroscopically and by their retention times relative to sitostanyl ferulate, and were quantified as previously reported (9). Rice and γ-oryzanol compounds were identified by mass spectrometry using authentic sterols standards as described (9). For each type of sample, each HPLC peak that was included in the calculations was further examined to verify that the respective spectra reasonably matched those of the major ferulate and *p*-coumarates. For corn samples, these were sitostanyl ferulate and sitostanyl *p*-coumarate; for rice, it was cycloartenyl ferulate. Some of the refined corn oils had retention times close to those of *p*-coumarate and ferulate of the crude oils, but the spectra were atypical, and thus those peaks were not included in the calculations. The yield of oil per unit of extracted source material is unknown for the commercial or process oils; therefore yields for bran samples are not given. Each bran sample was extracted twice, and two silica gel column preparations made for one of the extracts to assess within-sample reproducibility for the cleanup procedure. Quantitation and composition data are the averages of duplicate extraction analyses and of the single sample analysis. Two of each of the commercial oil samples (identified by source in the tables) were prepared and analyzed, and the averages are given. The average coefficient of variation (CV) within samples for the composition of the major components was 1.4% for sitostanyl ferulate, 2.4% for campestanyl ferulate, and 12.2% for sitosteryl *p*-coumarate. The average CV for the composition between samples was 1.9, 5.2, and 13.7%, respectively. The average CV for the quantitation within samples (oil basis) was 5.8, 5.5% between samples, and 3.0% for the amount of oil extracted.

RESULTS AND DISCUSSION

Analysis. Use of the Deltabond column gave better resolution of the esters in less time than the column used in previous work (9). The flexibility of the column is illustrated in Figure 1, showing that γ-oryzanol chromatographed with increasing amounts of water in the mobile phase. Similar results were obtained with the ester fraction from corn. Peak numbers 2, 3, 4, and 9 (17.5%) showed increasing resolution as the amount of water was increased; however, peak number 6 (12.5%) was gradually lost under these conditions. For samples of this ester composition, 12.5–15% water seemed optimal as all peaks can be seen at least as shoulders. Water at 13% gave the best resolution for corn esters; a typical chromatogram is shown in Figure 2. Three other C_{18} columns all gave results similar to those in Figure 1 using the base solvent system and run times from 30–70 min. However, there was better separation of peak numbers 8 and 9. Peak numbers 2, 3, and 4 appeared to be ferulates with an unidentified steryl portion. The Deltabond column offers considerable flexibility because a higher percentage of water can be used without substantially lengthening run times.

Composition and level of esters. The results of the analyses for corn materials are given in Table 1, and for rice in Table 2. Representative structures are shown in Scheme 1. Sample R, ConAgra corn bran flour, had been kept for over two years in a −20°C freezer; thus some deterioration may have occurred over time. A previous examination of this sample had given a value of 93.3 μg steryl CAD esters per gram of bran (9). The unrefined corn oils I and W were from the same manufacturer but had different labels on the bottles, similarly for samples U and V.

As is shown in Table 1, significant differences in the yields of esters from different brans and bran processing fractions were seen both on the basis of the amounts of esters per unit of bran as well as for the related yield for oil. The composi-

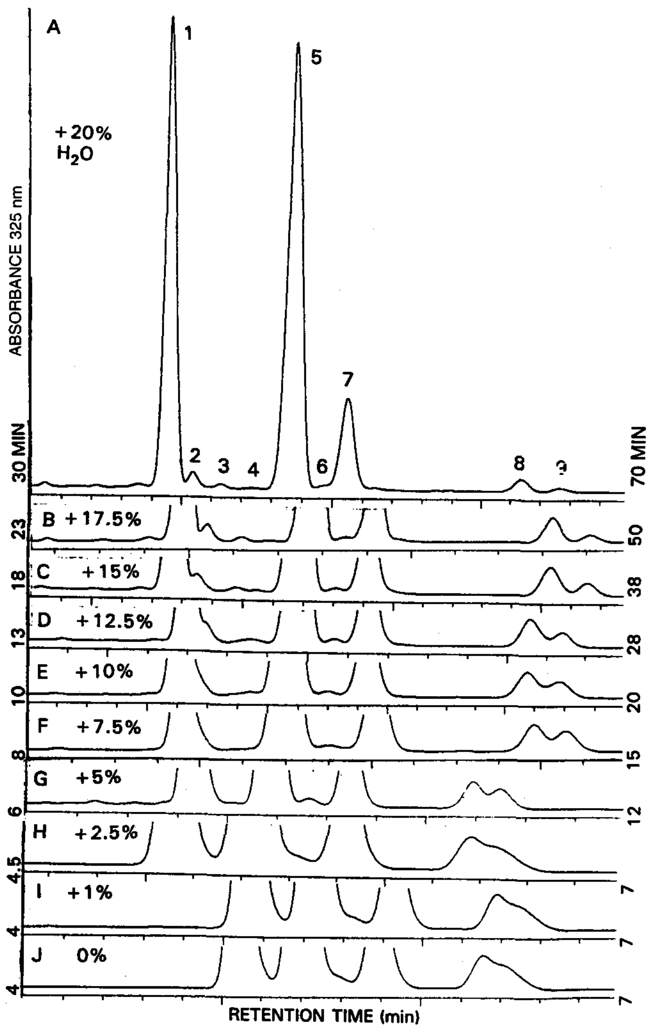


FIG. 1. Effect of increasing water concentration on the separation of γ-oryzanol components on a Deltabond C18 high-performance liquid chromatography column (Keystone Scientific, Bellefonte, PA) using acetonitrile/*n*-butanol/acetic acid/water (94:3:2:1, by vol) with additional water as shown. Detector wavelength, 325 nm. Peak 1, cycloartenyl ferulate; peaks 2, 3, 4, and 9, ferulates, sterol identity unknown; peak 5, 24-methylenecycloartanyl ferulate; peak 6, cyclobranyl ferulate; peak 7, campesteryl ferulate; peak 8, sitostanyl ferulate; not shown (off scale), cycloartanyl ferulate.

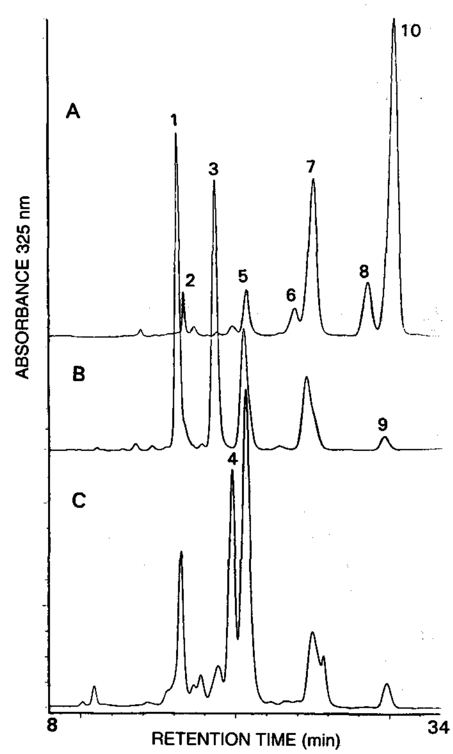


FIG. 2. Representative high-performance liquid chromatography chromatograms of the steryl ferulate and *p*-coumarate fractions of the corn and rice materials investigated. A. Ultra-high fiber bran. B. Rice bran extracted with hexane by author. C. Typical profile for commercial rice bran oils. Peak 1, cycloartenyl ferulate; peak 2, ferulate, sterol identity unknown; peak 3, 24-methylenecycloartanyl ferulate; peak 4, cyclobranyl ferulate; peak 5, campesteryl ferulate; peak 6, campestanyl *p*-coumarate; peak 7, campestanyl ferulate; peak 8, sitostanyl *p*-coumarate; peak 9, cycloartanyl ferulate; peak 10, sitostanyl ferulate.

TABLE 1
Steryl Ferulate and *p*-Coumarate Esters in Corn and Corn Oils[a]

		Oil[b] content	Total ester (mg ester/g)		Composition (wt%)					
Code	Source	(g)	[c]Bran	Oil	Δ^0-Sito fer	Δ^0-Campe fer	Δ^0-Sito *p*-cou	Δ^0-Campe *p*-cou	Other fer	Other *p*-cou
Corn bran and related fractions										
A	Expeller cake	2.51[d]	0.09	1.8	51.2	25.2	7.6	3.9	10.6	1.5
B	Raw bran	1.78[d]	0.22	6.3	63.5	27.9	1.1	0.4	6.8	0.3
C	Bran after duster	0.77[d]	0.24	15.8	65.5	24.4	1.8	0.7	7.0	0.6
D	Bran duster thrus	2.31[d]	0.54	11.5	62.1	27.2	1.4	0.5	8.3	0.5
E	Finished bran	0.93[d]	0.26	13.2	64.1	26.0	1.0	0.9	7.5	0.5
F	Ultra-high fiber bran	0.49[d]	0.23	23.9	69.4	21.6	1.6	0.6	6.5	0.3
G	Standard corn bran	0.77[d]	0.41	26.3	83.3	11.4	2.0	0.8	2.0	0.5
H	Fine high-fiber bran	0.75[d]	0.36	24.1	83.4	8.4	2.0	0.7	5.5	trace
R	Corn bran flour, ConAgra	0.88[d]	0.07	3.9	85.4	7.4	3.4	1.3	2.4	0.4
Corn oils, unrefined										
I	Spectrum Naturals	[e]		1.2	58.3	24.1	4.5	1.7	10.4	1.0
W	Spectrum Naturals	[e]		1.1	66.7	20.4	2.9	1.8	7.1	1.1
J	Lauhoff Grain Co.	[f]		8.6	59.3	27.3	2.0	0.9	9.9	0.6
K	Lauhoff Grain Co.	[e]		1.5	63.1	25.3	2.9	1.6	6.2	0.9
U	COOC, 32 oz	[e]		0.22	63.4	15.1	6.2	3.2	12.1	trace
V	California Naturals (COOC), 16 oz	[e]		0.18	64.0	22.9	2.3	0.8	9.1	0.9
Corn oils, refined										
S	Mazola	[g]	0.02	100	[h]—	—	—	—	—	
T	Hain Pure Food Co.	[e]	0.02	71.0	29.0	—	—	—	—	

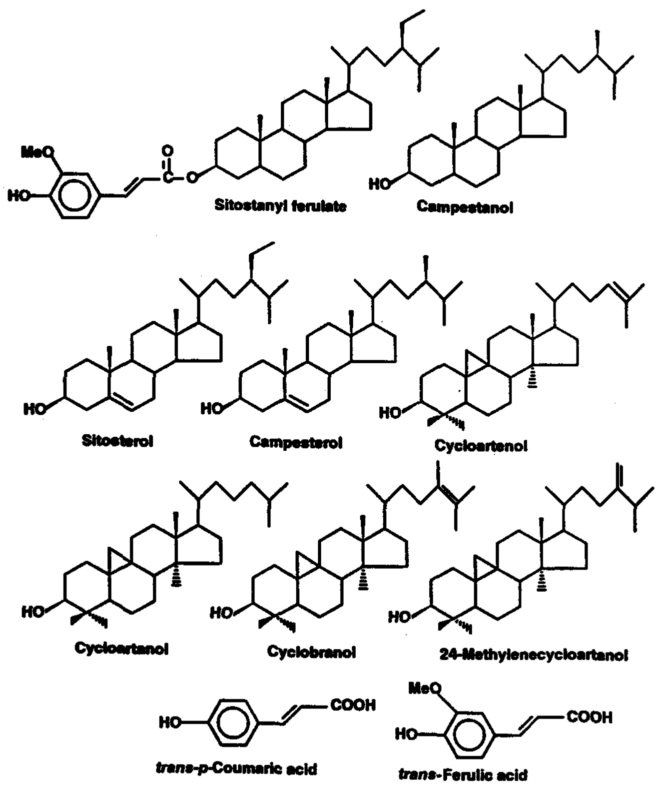
[a]Δ^0-Sito, sitostanyl; Δ^0-Campe, campestanyl; fer, ferulate; *p*-cou, *p*-coumarate; trace (<1%); COOC, The California Olive Oil Corp. (Salem, MA); ConAgra (ConAgra Grain Processing, Omaha, NE); Product A: Lauhoff Grain Co. (Danville, IL); Products B–H: Illinois Cereal Mills (Paris, IL); Spectrum Naturals (Petaluma, CA); Lauhoff Grain Co.; Mazola (CPC International, Inc., Englewood Cliffs, NJ); Hain Pure Food Corp. (Los Angeles, CA).
[b]Per 50 g (dry weight) bran.
[c]Dry weight basis.
[d]Hexane extracted by author.
[e]Expeller extracted by manufacturer.
[f]Hexane extracted by manufacturer.
[g]Extraction method unknown.
[h]Not quantified, interfering compounds.

TABLE 2
Steryl Ferulate Esters in Rice Bran and Rice Bran Oils[a]

		Oil[b] content	Total ester (mg ester/g)		Composition (wt%)					
Code	Source	(g)	[c]Bran	Oil	Cycloartenyl fer	24-methyleneCA fer	Cyclobranol fer	Sito fer	Campe fer	Other fer
N	Rice bran, NOW Foods	10.66[d]	3.4	15.7	29.4	28.9	trace	14.1	21.9	5.7
L	Rice bran oil, Select Origins	[e]		0.4	13.4	5.2	21.5	9.7	35.2	15.0
M	Rice bran oil, BioSan	[f]		3.0	27.4	4.8	20.1	10.6	24.2	12.9

[a]24-MethyleneCA, 24-methylenecycloartanyl; Sito, sitosteryl; Campe, campesteryl; fer, ferulate; trace, less than 2% of total; NOW Foods (Glendale Heights, IL); Select Origins (Southampton, NY); BioSan (New Dimensions Distributors, Fountain Hills, AZ).
[b]Per 50 g (dry weight) bran.
[c]Dry weight basis.
[d]Hexane extracted by author.
[e]Extraction method unknown.
[f]Expeller extracted by manufacturer.

tions of the different corn brans showed little variation except for samples G, H, and R which showed lower levels of campestanyl ferulate and corresponding increases in sitostanyl ferulate levels. There was an increase in the percentage of *p*-coumarates in the expeller cake; however, the unrefined corn oils, both expeller and hexane-extracted, were similar to the brans. The higher level of esters in the bran duster thrus fraction may be the result of an enrichment of this fraction in aleurone fragments which, since this layer is quite fragile by itself, would not be retained in the bran after duster fraction except as a part of a larger pericarp fragment. If the contribution of the endosperm to this fraction could be corrected for, the yield would be considerably higher. It was interesting that expeller cake had a significant level of esters.



SCHEME 1

The only previous study on the steryl CAD composition of different seed fractions was that of Seitz (1); however, the ester levels of the germ were not determined in that study. The presence of substantial amounts of esters in unrefined corn oils, both expeller and hexane-extracted, and in the cake remaining after expeller extraction, indicates that steryl CAD esters also occur in the germ as corn oil is extracted from the germ fraction of corn. Steryl ferulate esters have been found in corn germ oil (13) but their levels were not reported. Compared to bran and unrefined corn oils, the refined oils, samples S and T, contained only traces of the two major stanyl esters. Refined oils, such as S and T, are processed through several steps (including degumming, caustic refining, bleaching and deodorization), which apparently remove most of the esters. From 50–90% of the γ-oryzanol is removed in refining rice bran oil (7,11). Samples I/W and U/V showed that oils from the same source were similar in content but not identical.

Of particular interest is the effect of the method of extraction on the yield of esters from corn (Table 1). Samples J and K were from the same processor but were extracted by the expeller method for K, and by hexane extraction for J. Moreover, the amount of esters isolated in the latter case was 553% greater. The levels found in expeller oils from samples I and W were similar to those of K, but two other expeller oils (U,V) were considerably lower. The effect of the extraction method showed a similar pattern in rice bran oils (Table 2). No commercial sample of unrefined rice bran oil identified as hexane-extracted was available, but bran extracted by us in the laboratory (N) showed a similar 530% higher level over that of the highest commercial oil examined (M). The other sample (L) had apparently been subject to additional refining steps. There was a marked difference in the levels of two components in the rice bran oils vs. γ-oryzanol and rice bran freshly extracted: cyclobranol was present only in trace amounts in γ-oryzanol and bran; however, its level was markedly higher in the rice bran oils examined. The level of 24-methylenecycloartanyl ferulate in the oils decreased proportionately (Table 2). The apparent health benefits of γ-oryzanol have led to increasing interest examining the steps in rice bran oil refining which affect the levels of γ-oryzanol (11), and in alternate processing modes which would retain these components in the finished oil product (14). The values reported here for rice bran and for the oil extracted from it are similar to the γ-oryzanol levels previously reported for rice bran and oil (8,11). The total ester levels of the two commercial oils were also similar to levels previously reported (7,15). Tables 1 and 2 show that the level of steryl ferulate esters in the oil from rice bran was lower than the level in oil from some types of corn bran; however, because of the much higher oil content of rice bran, the yield of esters on a bran basis would be six to eight times higher.

The effect of rice bran, rice bran oil, and γ-oryzanol on cholesterol metabolism has been the subject of several studies which have recently been reviewed (7). Similar studies seem not to have been carried out on the effect of nonrice steryl CAD esters on cholesterol uptake or serum cholesterol levels. As shown in Table 1, ferulate ester of sitostanol accounts for 65–85% of the ester fraction in oil from corn bran. Sitostanol has been reported to be as effective as sitosterol, at 10-fold lower levels, in lowering serum cholesterol in hypercholesterolemic patients (16), and it was suggested that low-dose sitostanol might be a useful hypolipidemic agent for the treatment of mild hypercholesterolemia. Fatty acid esters of cholesterol and of plant sterols are known to be hydrolyzed in the course of fat digestion (17,18). The effectiveness of plant sterols in lowering blood cholesterol levels is thought to result from their ability to displace free cholesterol from intestinal micelles (18). Rukmini and Raghuram (19) reported that cycloartenol is as effective as γ-oryzanol in lowering serum cholesterol in rats, which would be consistent with the idea that cycloartenyl ferulate is hydrolyzed and that the released sterol has an effect similar to that of the sterol taken up in the diet. Sitostanol has a further advantage in that it is not appreciably taken up into the bloodstream (16,20). The limited solubility of sitostanol in fats, however, has made it difficult to administer it (21), and it is only when plant sterols are suspended in lipids that they can be administered at a dose high enough to be effective (18,21). A recent report has shown that the effectiveness of sitostanol was markedly increased when the sterol was administered as a fatty acid ester with the dietary fat (21). Given the high percentage of sitostanol esters in the steryl CAD ester fraction of corn bran oil and the levels of esters comparable to or higher than the ester levels in rice bran oil, it would seem that further studies on the effect of these compounds on blood cholesterol levels would be needed. The approaches presented in this paper should prove helpful in screening cereals for sterol esters of cinnamic acid derivatives and in selecting the appropriate corn fraction or oil for the isolation of steryl ferulate and *p*-coumarate esters or for feeding studies with crude preparations.

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